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Substrate complexity reduces prey consumption in functional response experiments: Implications for extrapolating to the wild

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Abstract

Understanding the density-dependent impacts of an invasive predator is integral for predicting potential consequences for prey populations. Functional response experiments are used to assess the rate of prey consumption and a predator's ability to search for and consume prey at different resource densities. However, results can be highly context-dependent, limiting their extrapolation to natural ecosystems. Here, we examined how simulated habitat complexity, through the addition of substrate in which prey can escape predation, affects the functional response of invasive European green crabs (*Carcinus maenas*) foraging on two different bivalve species. Green crabs feeding on varnish clams (*Nuttallia obscurata*) shifted from a Type II hyperbolic functional response in the absence of substrate to density-independent consumption when prey could bury. Green crabs ate few Japanese littleneck clams (*Venerupis philippinarum*) under all densities, such that no functional response curve of any type could be produced and their total consumption was always density independent. However, the probability of at least one Japanese littleneck clam being consumed increased significantly with initial clam density and crab claw size across all treatments. At mean crab claw size and compared to trials without substrate, the proportion of varnish clams consumed were 4.2 times smaller when substrate was present, but substrate had a negligible effect (1.2 times) on Japanese littlenecks. The proportion of varnish clams consumed increased with crab claw size and were higher across both substrate conditions than the proportion of Japanese littlenecks consumed; however, the proportion of Japanese littleneck clams consumed increased faster with claw size than that of varnish clams. Our results suggest that including environmental features and variation in prey species can

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influence the density-dependent foraging described by functional response experiments. Incorporating replicable features of the natural environment into functional response experiments is imperative to make more accurate predictions about the impact of invasive predators on prey populations.

Keywords
Decapods, density-dependent predation, environmental complexity, invasive species, marine, non-native

Introduction

Owing to new introductions and ever-expanding ranges, invasive species have significant negative impacts on the biodiversity (McNeely 2001; Molnar et al. 2008; Mollot et al. 2017), trophic structure (Nilsson et al. 2012; Papacostas and Freestone 2019) and overall integrity of ecosystems (Miehls et al. 2009; Walsh et al. 2016). Marine invasions, in particular, have significant economic and ecological consequences, with coastal marine ecosystems being amongst the most invaded due to the multiplicity of co-occurring anthropogenic activities (e.g. commercial shipping, marine aquaculture etc.) (Simberloff 2013; Geburzi and McCarthy 2018; Cuthbert et al. 2019; Pyšek et al. 2020). The ever-changing seascape of invasions makes it challenging to accurately predict the potential impacts of non-native species on ecosystems and hampers effective management (Beardsell et al. 2021).

The population density of an invader is important for estimating its potential impact and the resulting consequences for the environment (DeRoy et al. 2020a; Griffen et al. 2021). The influence of invasive species on an ecosystem is often density dependent, as it is linked to both the per-capita effect of an invader and invader abundance (Parker et al. 1999; DeRoy et al. 2020a). Functional response experiments (FREs) are often used in invasion ecology to assess the rate of resource consumption, usually in the context of a predator's ability to search for and consume prey at different resource densities (Holling 1959; Alexander et al. 2012; Beardsell et al. 2021). The resulting functional response (FR) curve can reveal whether predators could have a linear (Type I), destabilising (hyperbolic; Type II) or stabilising (sigmoidal; Type III) effect on prey populations (Holling 1959), which is important for forecasting the impact of new invaders.

Functional response experiments, like many laboratory experiments, are by nature simplified representations of complex systems. They typically remove many of the biotic and environmental variables that may influence consumption rates, increasing the comparability of findings within and between species (Holling 1959; Dick et al. 2014; Beardsell et al. 2021). However, invasion dynamics can be highly context-dependent, with every invasion into a novel environment resulting in different relationships between the invader and the surrounding system. Environmental conditions and biological interactions, including temperature, prey species and the sex of the predator (Lipcius and Hines 1986; Sponaugle and Lawton 1990; Beardsell et al. 2021; Howard et al. 2022; Kattler et al. 2023), can impact the shapes and asymptotes of FR curves.

Therefore, the factors included or excluded from the design of a FRE can be integral for the interpretation of the impact of that specific invader. The incorporation of a variable, such as habitat complexity (e.g. by adding substrate into which prey can hide), in laboratory experiments has been shown to be a determining factor in the relationship between predators and prey (Barrios-O'Neill et al. 2014; Barrios-O'Neill et al. 2016; DeRoy et al. 2020b). It can increase search effort, shift the profitability of different prey sizes and alter consumption rates (Sponaugle and Lawton 1990; Barrios-O'Neill et al. 2014; Barrios-O'Neill et al. 2016; DeRoy et al. 2020b). The addition of such a variable to a FRE could be important for understanding the context dependency of novel invasive predator-prey relationships.

Functional response experiments have been used to evaluate the predatory behaviour and potential impacts of the European green crab (*Carcinus maenas*, Linnaeus, 1758) (e.g. Howard et al. 2019; Ens et al. 2021; Howard et al. 2022). Native to Europe and northern Africa, green crabs are now found on every continent but Antarctica (Yamada 2001; Klassen and Locke 2007; Young and Elliott 2019) and are one of the most studied invasive species globally (Watkins et al. 2021). They are generalist predators (Yamada 2001; Klassen and Locke 2007) and have been shown to have significant negative effects on invaded ecosystems through predation (Whitlow et al. 2003; Miron et al. 2005), competition (MacDonald et al. 2007) and habitat alteration (Malyshev and Quijón 2011; Howard et al. 2019). In particular, green crabs are known to be highly effective predators of clams and have had substantial negative consequences for bivalve fisheries across the globe (Ropes 1968; Klassen and Locke 2007; Whitlow 2010). The worldwide success of green crabs is due to their ability to tolerate a wide range of environmental conditions and their broad diet (Yamada 2001; Lohrer and Whitlatch 2002; Klassen and Locke 2007; Young and Elliott 2019). These wide physiological limits and patterns of resource use make any predictions about the impact of green crabs extremely context-specific as the species' behaviour may be similar (Howard et al. 2019) or different across novel ecosystems (Howard et al. 2018). Therefore, incorporating realistic environmental elements relevant to the invasion being studied to FREs on green crabs could provide more context-specific estimates of their potential effects on prey populations.

In this study, we aimed to examine how the FR of invasive green crab foraging on bivalve species may change when prey are provided with habitat that mimics their natural environment. More specifically, we provided substratum in which bivalve prey could bury, thereby potentially increasing green crab handling time and decreasing their attack rate and maximum prey consumption when compared to FREs conducted without substrate. We also examined the effect of prey species characteristics that can impact susceptibility to predation, i.e. morphological characteristics and burial depths, in these two substrate conditions. We expected that the different burying depths of the two clam species used might give rise to a reversal in prey profitability that foraging crabs would experience in the wild, but may not be realised in typical FREs. Increasing search time in a FRE could alter predictions of the magnitude of impact invasive predators have on prey populations.

Methods

Study species and collection

Male European green crabs were collected from Bedwell Bay (49°18.55'N, 125°48.29'W) near Tofino on the west coast of Vancouver Island, British Columbia (BC), Canada, in June 2022. Crabs without evidence of moulting, free from epibionts, with a notch-to-notch carapace width of 55 to 76 mm and with both chelipeds present, were used in our experiment. We collected only males to reduce the risk of invasion via the release of fertilised eggs at the experimental facility. Varnish clams (*Nuttallia obscurata* Reeve, 1857) were collected from Robbers Passage (48°53.77'N, 125°7.25'W) in Barkley Sound, also on the west coast of Vancouver Island, while Japanese littleneck clams (*Venerupis philippinarum* A. Adams & Reeve, 1850) were collected from Nanoose Bay (49°15.53'N, 124°10.99'W), on the east coast of Vancouver Island. We collected clams with undamaged shells and measuring 21–40 mm in length (i.e. anterior to posterior shell margins).

Both prey species are not native to BC, but their high abundance in soft-bottom habitats throughout the region results in a high likelihood of encounters between these invasive prey and green crabs (Quayle 1964; Gillespie et al. 2001; Dudas et al. 2005; Blackburn et al. 2011). Varnish clams are a prevalent clam species found in the Salish Sea (Gillespie et al. 2001). They possess similar physical characteristics to the native bent-nosed macoma (*Macoma nasuta*) (Dudas et al. 2005; Hiebert et al. 2015; Gordon 2018) and are found at similar depths and tidal heights, burying to around 10–20 cm (Gordon 2018). The second invasive prey species used here, Japanese littleneck clams (or Manila clams), are also abundant and similar in morphology and habitat use to the native Pacific littleneck clams (*Protothaca staminea*) (Richardson 1985), to such an extent that native crabs appear to be unable to distinguish between them (Dudas et al. 2005). Due to their short siphons, both species of littleneck clams bury to depths shallower than 10 cm (Richardson 1985). These ecological similarities between varnish clams and Japanese littleneck clams with their native counterparts, as well as their widespread occurrence and abundance in coastal BC, led to our decision to use invasive prey species for our experiment.

All animals were held at the Bamfield Marine Science Centre, on the west coast of Vancouver Island, in indoor sea tables (172 cm long × 75 cm wide × 16 cm deep) with flow-through, unfiltered seawater (10 °C ± 0.33 °C). The animals were held under artificial lighting that mimicked natural day-night cycles. Crabs were held at low densities (~20 crabs per sea table) with ample habitat including flowerpots, rocks, PVC pipes and seaweed. Crabs were fed thawed salmon pieces every four days. Clams were fed algae and Phytofeast every three days.

Experimental set-up

Functional response experiment

Trials were conducted in opaque plastic enclosures (61 cm × 41 cm × 42 cm), which were all supplied with natural seawater flowing at equal rates. Each replicate consisted

of 12 treatment combinations: two substrate treatments (enclosures with or without substrate) at each of six clam densities (1, 2, 4, 6, 10 or 16 individuals per enclosure). We placed Quikrete® premium play sand on the bottom to a depth of 20 cm in each with-substrate enclosure and left the bottom of the no-substrate enclosures bare. We chose to use play sand instead of natural substrate to avoid variability introduced by grain size, the possible presence of invertebrates and/or variation in oxygen levels in natural substrate. Sand was washed thoroughly before use and the seawater used in the experimental enclosures was changed and all visible detritus removed between trials. Each trial included a single clam species; there were no mixed-species trials. All 12 treatment combinations were replicated six times each for both varnish clams and Japanese littleneck clams (for a total of 144 trials). Each replicate was run over two days between 25 June and 9 July 2022. Clam density by substrate level combinations were randomly assigned to each enclosure using a random number generator. Clams were scattered in the enclosures 12 h prior to the start of each replicate and were only used once, even if they were not consumed. Each trial commenced with the introduction of a single, randomly-assigned crab. Prior to trials, we isolated and withheld food from green crabs for 48 h to standardise hunger levels (Howard et al. 2018, 2022). Each crab was used only once. Before each trial, we recorded morphological characteristics of the green crabs and both clam species, as these could influence both the ability of crabs to open a clam and the profitability of individual clams as prey (Elner 1980; Yamada 2001; Byers 2002; Dudas et al. 2005). We measured crab carapace width (CW) from notch to notch at the widest part of the carapace and cheliped height (CH) as the maximum height of the major cheliped propodus (top to bottom). We also measured clam length with calipers. Crabs were allowed to forage for 8 h beginning at 08:00 h.

At the end of each trial, crabs were removed and the number and size of clams consumed were recorded. Finally, we ran a control replicate to test for clam survival independent of predation. Temperature and salinity were measured at the start and end of each trial using a thermometer (Fisherbrand™ 76 mm immersion thermometer) and refractometer (Tropic Eden PRO-1 normal seawater refractometer), respectively.

Burial depths of prey species

We ran a burial experiment to determine the average burial depth of both clam species. We glued a graduated length of monofilament fishing line to the umbo of 15 clams of each species and allowed them to bury in identical substrate conditions as the FR experiment for 12 h. After 12 h, we measured each line from the umbo of the clam to the point where the line emerged from the sand.

Statistical analysis

For each treatment combination, we attempted to fit a functional response curve to the proportion of prey eaten in relation to prey density using the R package 'frair' (frair:frair_test) (Pritchard et al. 2017). We first used the 'frair_test' function, which compares two logistic regressions on the proportion of prey consumed: one that looks

at the effect of density (i.e. representing a Type II relationship) and the other that looks at the effect of density and density squared (i.e. representing a Type III relationship) (Pritchard et al. 2017). The function uses significance testing to determine if there is sufficient evidence to fit a functional response curve. A Type III response is supported when both the density and density-squared terms are significant. When the density term is significant and negative, but density squared is not, a Type II response is supported. In the case where neither term is significant, the test offers no support for the existence of either type of response. The only treatment combination that had a significant term from this initial logistic regression was that of green crabs feeding on varnish clams in the absence of substrate (see Results). The first-order terms were negative, indicating a Type II FR. Therefore, we used the random predator equation without prey replacement (Rogers 1972):

$$N_e = N_0 (1 - \exp(a (N_e h - T)))$$

where N_e is the number of prey eaten, N_0 is the starting prey density, a is the predator's attack rate, h is the handling time and T is the length of the experiment. N_e and N_0 were determined by each individual trial, while a and h were estimated from the logistic regression model. We then used frair:frair_boot non-parametric stratified bootstrapping (n = 2000 iterations) to generate a 95% confidence interval for each parameter estimate of the model. We used a bias-corrected and accelerated bootstrap interval (upper and lower BCa) to correct for any biases or skewed distributions in the bootstrapped model. There was no evidence of a functional response for any of the other treatment combinations (i.e. none of the terms was significant for the initial logistic regression), so we therefore did not generate FR equations and the associated parameters (see Results).

As the logistic regression used by the 'frair_test' did not produce any significant density terms for three of our treatment combinations, we used an additional approach to understand the role of substrate presence in green crab foraging by considering other possible explanatory variables in addition to prey density. We first ran a separate logistic regression (generalised linear model with a binomial distribution and logit link function) to examine the probability that a crab consumed a clam in relation to cheliped height, initial clam density and substrate presence, as well as interactions between clam species and substrate presence/absence and between clam species and cheliped height. However, complete separation (i.e. one variable perfectly predicts another variable) occurred in the model for varnish clams in the absence of substrate treatment. This was caused by every crab consuming at least one varnish clam in every no-substrate trial. Therefore, we incorporated bias reduction through a maximum penalised likelihood for our observations where penalisation was done using Jeffreys invariant prior (Kosmidis 2021).

We then used a second logistic regression (generalised linear model with a binomial distribution and logit link function) to assess the proportion of clams consumed in a trial in relation to the same variables and interactions as the previous model. In both models, the interaction between clam species and substrate presence/absence was included to reveal the potential trade-off between attack rate and handling time generated

by the different burial depths of the two clam species (Richardson 1985; Gordon 2018). The interaction between clam species and cheliped height was included to reflect the potential effect of clam species differences (especially in shell thickness) on the relationship between in cheliped height and consumption (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992). All analyses were done using R version 4.2.2 (R Core Team 2022). We analysed model fit using the 'DHARMa' package (Hartig 2022) to assess the distribution of residuals, dispersion, outliers and variances for our models. We fit our generalised linear model with bias reduction using the 'brglm' package (Kosmidis 2021).

Results

There was no mortality for either varnish clams or Japanese littleneck clams in substrate and non-substrate trials when in enclosures without green crabs. Therefore, all mortality observed in the experiment was assumed to be due to green crab predation. All clams were able to bury themselves before the start of each trial. Varnish clams buried significantly deeper (mean ± 1 SE: 8.98 ± 0.48 cm, range: 6.2–11.6 cm) than Japanese littleneck clams (4.15 ± 0.29, 2.3–6.2 cm) (coefficient = 4.83, $p < 0.001$, Suppl. material 1: fig. S1).

The lengths of varnish clams consumed ranged from 25 to 40 mm (mean ± 1 SE: 31.09 ± 0.30 mm), while Japanese littleneck clams that were consumed ranged from 21 to 30 mm (mean ± 1 SE: 26.79 ± 0.35 mm). There was no significant difference between the sizes of varnish clams that were or were not consumed, irrespective of substrate presence (post-hoc pairwise contrasts not consumed vs. consumed, with substrate: estimate = -0.47, $t_{691} = -0.94$, $p = 0.35$; without substrate: estimate = -0.19, $t_{691} = -0.43$, $p = 0.67$; Suppl. material 1: fig. S2). For Japanese littleneck clams in the absence of substrate, consumed clams were on average 1.68 mm smaller than those that were not consumed (post-hoc pairwise contrast, $t_{461} = 2.93$, $p = 0.0035$). However, when substrate was present, there was no significant difference in the size of clams consumed (estimate = 0.62, $t_{461} = 0.94$, $p = 0.35$).

Functional responses

Green crabs feeding on varnish clams in the absence of substrate exhibited a Type II hyperbolic FR, with a corresponding significant negative first-order density term ($z = -4.57$, $p < 0.001$, Fig. 1; Table 1). Due to the non-significant logistic regressions, we did not fit functional responses to the data from the other three treatment combinations (Suppl. material 1: fig. S3). The non-significant regressions are likely due to the number of trials where a crab did not consume any clams, resulting in a sample size too small to analyse (0/36, 24/36, 26/36 and 26/36 crabs did not eat varnish clams in the absence of substrate, varnish clams with substrate, Japanese littleneck clams in the absence of substrate and Japanese littleneck clams with substrate, respectively), especially at the lower prey densities (Pritchard et al. 2017).

Table 1. Parameter estimates for green crabs feeding on varnish clams in the absence of substrate. The parameter estimates, attack rate (a) and handling time (h), were derived from a Rogers Type II functional response curve. BCa CI represents the bootstrapped accelerated bias-corrected 95% confidence intervals.

Parameter	Estimate	SE	BCa CI	*z*	*p*
Attack rate (a)	2.89	0.77	1.72–5.59	3.74	< 0.01
Handling time (h)	0.12	0.02	0.06–0.17	5.79	< 0.01

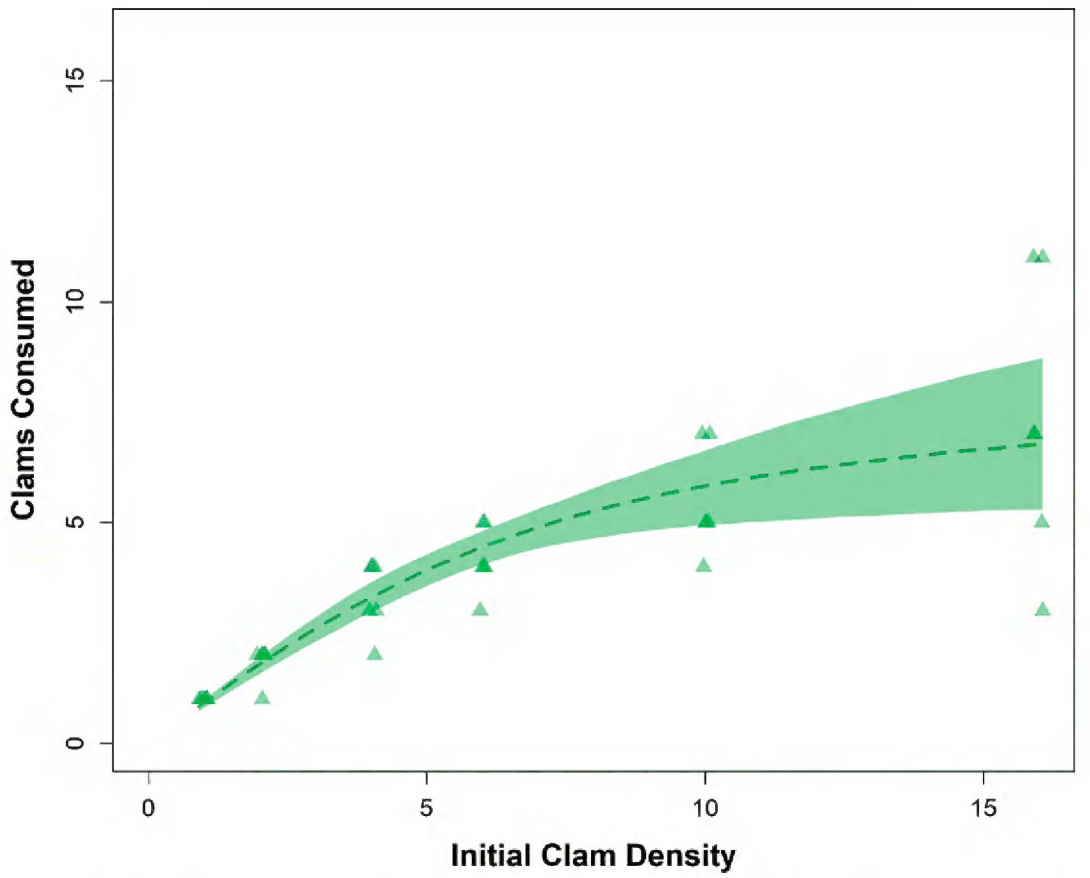

Figure 1. Functional response curve of green crab feeding on varnish clams in the absence of substrate. The triangles show the mean number of varnish clams consumed as a function of initial clam density (1, 2, 4, 6, 12 or 16 per trial). The Type II FR curve is represented by the dashed line with the bootstrapped 95% confidence interval represented by the shaded ribbon.

Drivers of predator consumption

The probability of an individual clam being consumed increased with cheliped height and initial clam density across all treatment combinations considered (Table 2). In the absence of substrate, the probability that green crabs would consume at least one clam was significantly higher for varnish clams than for Japanese littlenecks (coefficient = 6.48, p = 0.05). The probability that at least one Japanese littleneck clam was consumed was similar whether there was substrate or not when all other variables were constant (Table 2). However, there was a significant interaction between clam species and substrate presence. In the absence of substrate, green crabs were 10.84 times more likely to consume at least one varnish clam than at least one Japanese littleneck clam when the initial clam density was one. In contrast, in the presence of substrate, the probability of at least one varnish clam being consumed was only 1.28 times greater than that of Japanese littleneck clams when the initial clam density was one (Fig. 2, Table 2).

Table 2. Results of a generalised linear model (GLM) with bias reduction, binomial distribution and logit link function examining the effect of various factors on the probability that a green crabs would consume at least one clam during a trial. Substrate refers to the presence or absence of substrate in an enclosure, initial clam densities were 1, 2, 4, 6, 12 or 16 clams and clam species included varnish clams or Japanese littleneck clams. The baseline factor levels for the model are Japanese littleneck clams in the absence of substrate (n = 144 trials).

Factor	Estimate	SE	*z*	*p*
Intercept	-6.91	1.79	-3.86	< 0.001
Cheliped height	0.24	0.083	2.95	0.003
Initial clam density	0.19	0.049	3.88	< 0.001
Clam species	6.48	3.37	1.93	0.05
Substrate	-0.096	0.62	-0.16	0.87
Clam species × substrate	-5.93	1.73	-3.43	< 0.001
Clam species × cheliped height	-0.015	0.18	-0.085	0.99

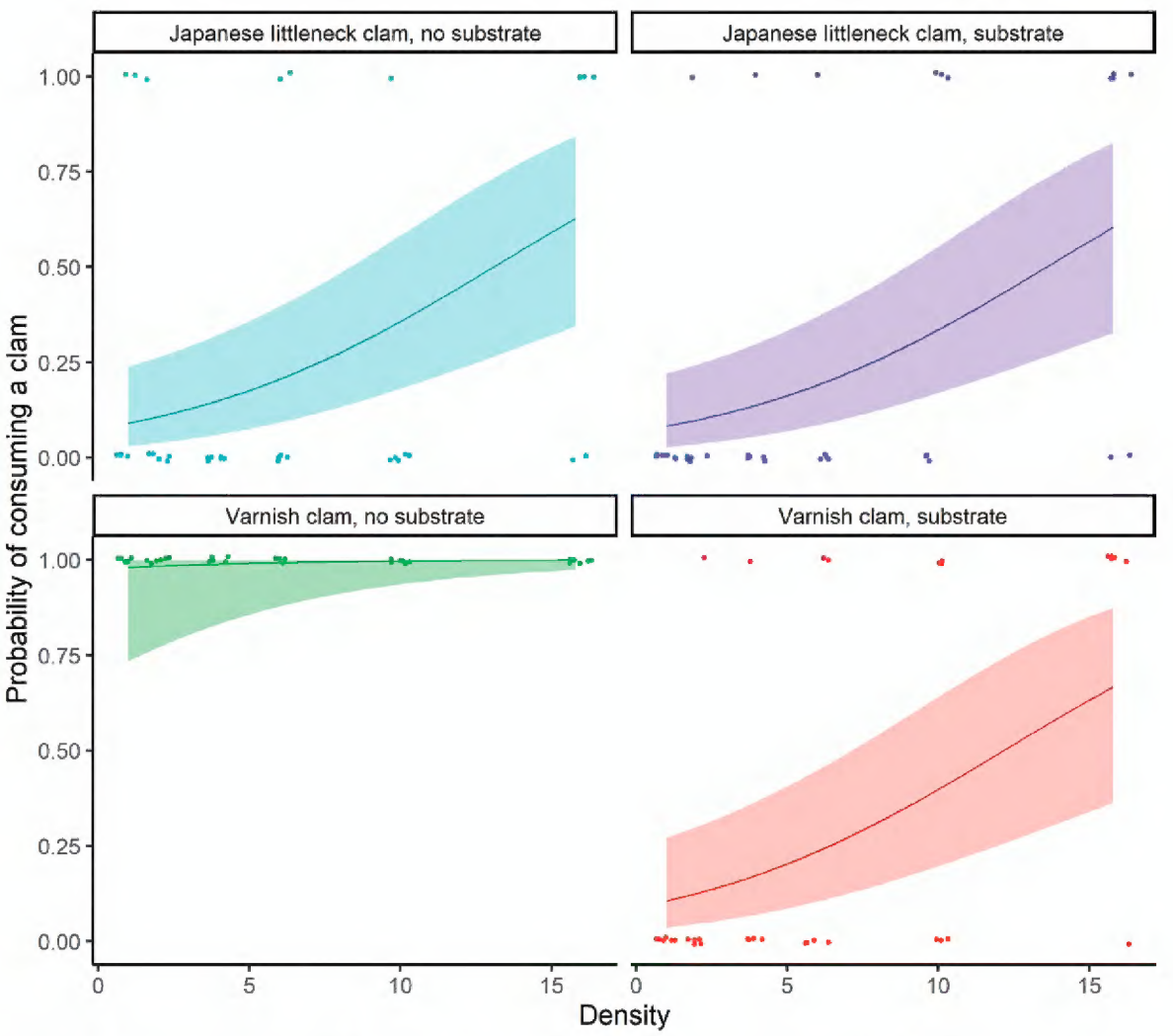

Figure 2. Probability of a green crab consuming a clam as a function of initial clam density. Lines represent model predictions for each treatment combination (varnish clams or Japanese littleneck clam in the presence or absence of substrate) during a trial in relation to initial clam density and ribbons represent 95% confidence intervals. Data points represented individual crabs (n = 36 for each density x substrate treatment).

This trend continues at higher initial clam densities. For example, when 10 clams were initially present, varnish clams had a 2.79 times higher probability of at least one clam being consumed in the absence of substrate than Japanese littleneck clams, but only

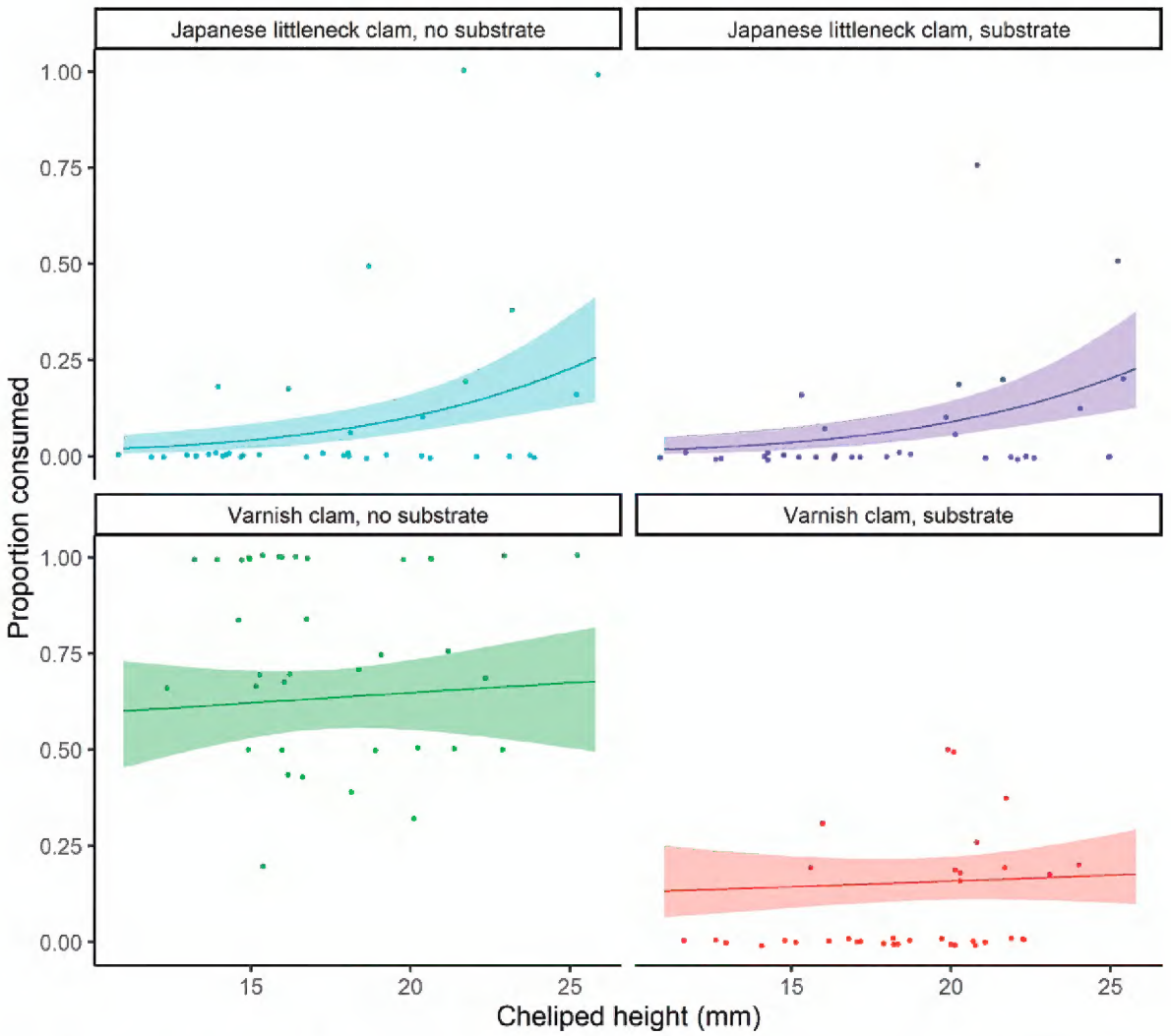

Figure 3. Proportion of varnish clams or consumed in relation to green crab cheliped height (mm). Lines represent model predictions for each treatment combination (varnish clams or Japanese littleneck clam in the presence or absence of substrate) and ribbons represent 95% confidence intervals. Data points represented individual crabs (n = 36 for each density x substrate treatment).

1.19 times higher probability when substrate was present (Fig. 2, Table 2). There was no significant interaction between clam species and crab cheliped height (Table 2). Overall, the probabilities of clam consumption by green crabs feeding overlapped extensively across all treatment combinations, with the exception of varnish clams in the absence of substrate (Fig. 2).

Crab cheliped height, clam species and the interactions between clam species and substrate presence and between clam species and cheliped height all had a significant effect on the proportion of clams consumed during a trial (Table 3). The proportion of clams consumed increased weakly, but non-significantly, with cheliped height in both substrate conditions (Fig. 3). There was a significant negative interaction between cheliped height and clam species (Table 3). At the mean cheliped height of 18.08 mm, the proportion of Japanese littleneck clams consumed increased by 1.19 times with a one-mm increase in cheliped height in both the absence and presence of substrate (Fig. 3). The proportion of varnish clams consumed only increased by 1.008 times with a one-mm increase above the mean cheliped height in the absence of substrate, but by 1.02 times in the presence of substrate (Fig. 3). While the presence of substrate had no significant effect on the proportion of clams eaten overall, there was a significant negative interaction between clam species and the presence of substrate (Table 3).

Table 3. Results of a generalised linear model (GLM) with a binomial distribution and logit link function examining the effect of various factors on the proportion of clams consumed during a trial as a function of green crab cheliped height. Substrate refers to the presence or absence of sand in an enclosure, initial clam densities were 1, 2, 4, 6, 12 or 16 clams and clam species included varnish clams or Japanese littleneck clams. The baseline factor levels for the model are Japanese littleneck clams in the absence of substrate (n = 144 trials).

Factor	Estimate	SE	z	p
Intercept	-5.81	1.051	-5.53	< 0.001
Cheliped height	0.19	0.049	3.87	< 0.001
Initial clam density	-0.0305	0.018	-1.66	0.10
Clam species	6.11	1.26	4.84	< 0.001
Substrate	-1.15	0.36	-0.42	0.67
Clam species × substrate	-2.13	0.43	-4.92	< 0.001
Clam species × cheliped height	-0.17	0.064	-2.62	0.009

Specifically, while the proportion of Japanese littleneck clams consumed was similar in trials with and without substrate (increased by 1.13 times at the mean cheliped height in the absence of substrate), the mean proportion of varnish clams consumed was 4.19 times higher without than with substrate at the mean cheliped height (Fig. 3). Overall, the proportion of Japanese littlenecks consumed was significantly lower than the proportion of varnish clams consumed (Table 3, Fig. 3).

Discussion

European green crabs did not always forage on clams in the density-dependent manner described by functional responses. Green crabs feeding on varnish clams in the absence of substrate consumed prey in every trial and exhibited a Type II hyperbolic FR, indicating a potentially destabilising effect on this prey species at low densities. However, green crabs in the other three treatment combinations (varnish clams in substrate and Japanese littleneck clams with and without substrate) consumed too few clams to exhibit a significant density term to support a density-dependent Type II or III response. The probability of a crab consuming at least one prey increased with prey density and crab crusher claw size and prey species interacted with substrate condition. A lower proportion of varnish clams were consumed in trials with than without substrate, but no difference was detected for Japanese littleneck clams. Our findings suggest that the results of FREs and, hence, the conclusions drawn about the potential effect of predators on wild populations, are heavily influenced by their experimental simplicity.

We had originally expected that the addition of substrate in our experiments would alter the shape and/or asymptotes of the resulting FR curves. More specifically, we had predicted that search time and handling time might increase and maximum prey consumption might decrease, when substrate was present. We had also expected that adding substrate might reverse the profitability of the two clam species due to their contrasting features (i.e. differences shell thickness and burial depth) (Byers 2002; Dudas et al. 2005) and, hence, transpose their FR curves. These predictions were only partly supported. Consumption of

varnish clams by green crabs was lower in the presence than in the absence of substrate, as expected. When varnish clams could bury, most green crabs failed to eat, such that we could not reliably model a FR curve and associated parameters as the resulting confidence intervals would cross zero. This was also true for Japanese littlenecks in the presence and absence of substrate. In the absence of substrate, all green crabs consumed varnish clams, while fewer than half consumed Japanese littlenecks, which indicates a likely preference for the former prey species. This preference in the absence of substrate may be linked to shell robustness (Boulding 1984), since green crabs were unselective when foraging on varnish clams, but selected Japanese littleneck clams that were smaller and, hence, easier to crush, than the ones that remained uneaten (Suppl. material 1: fig. S2). However, clam size is no longer a factor when crabs have to dig to access buried prey. Our results, therefore, suggest that density-dependent foraging, as described by functional response theory, occurs only when green crabs forage on preferred prey and search time is minimised by the absence of habitat complexity. The fact that both environment and prey species can significantly alter the consumption rate of a predator reveals the importance of experimental design choices (Lipcius and Hines 1986; Rossi et al. 2008; Howard et al. 2018).

Density-dependent foraging still occurred in the three treatment combinations where functional responses were not supported. For varnish clams in the presence of substrate and Japanese littleneck clams in both substrate conditions, the probability of a green crab consuming a clam increased significantly with both prey density and crab cheliped height. In general, the more abundant the prey, the higher the likelihood of a prey encounter, even when prey are concealed (Sponaugle and Lawton 1990; Ebersole and Kennedy 1995; Seitz et al. 2001). In addition, the consumption rates of most decapod crustaceans are typically limited by cheliped height as it can determine both the prey species and size that they can consume (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992; Dudas et al. 2005). However, while there was no significant interaction between crab cheliped height and clam species, green crabs were 1.5 to 9.2 times more likely to consume at least one varnish clam in the absence of substrate than in the presence of substrate, with the magnitude of the effect varying inversely with initial clam densities. The same pattern can be seen with Japanese littleneck clams, though to a lesser extent (1.04 to 1.09 times more likely in the absence than presence of substrate). For varnish clams, which are thin-shelled but bury deep (Byers 2002; Dudas et al. 2005), the probability of at least one clam being consumed in a trial fell from 100% in the absence of substrate to overlap almost completely with that of Japanese littlenecks when substrate was present. In contrast, Japanese littleneck clams, which are thick-shelled and bury shallow (Byers 2002; Dudas et al. 2005), had the same probability of being eaten in both substrate treatments. Therefore, adding substrate appeared to reduce the profitability of the preferred varnish clam species to a level similar to that of the less preferred Japanese littleneck.

The drivers of the proportions of clams consumed support our interpretation of the previous results. While cheliped height did not determine the probability of a crab consuming a clam, the proportion of clams eaten increased with cheliped height, as expected (Elner 1980; Hamano and Matsuura 1986; Lee and Seed 1992; Dudas et al. 2005), across all treatment combinations. The ability of a green crab to open a thin-shelled varnish clam does not depend as strongly on cheliped height as the ability to crack open

a thicker-shelled littleneck. This appears to be also true for the co-occurring native crab species red rock (*Cancer productus*) and Dungeness crabs (*Metacarcinus magister*) which, in the absence of substrate, consume a wide range of varnish clam sizes but a more limited size range of littleneck clams (Dudas et al. 2005). These results suggest that the extra time and energy required of green crabs to forage on varnish clams in more 'natural' substrates are substantial burdens on detection and attack rates, resulting in lowered consumption. Such burdens on green crab consumption are not evident when prey are associated with long handling times, such as thick-shelled littlenecks.

We expect that the relatively low temperature of our experiments contributed to some of the low consumption rates observed, particularly if thermal effects on foraging are prey-specific (e.g. more important when more crushing force is required). It is well documented that European green crabs consume more prey as temperatures increase (Ropes 1968; Elner 1980; Howard et al. 2022) and are less active at colder temperatures (Yamada 2001; Young and Elliott 2019). However, the experimental temperatures experienced by our green crabs (10 °C ± 0.33 °C) were a few degrees above the minimum temperature for green crab feeding (7 °C) (Yamada 2001) and green crabs did consume prey in all treatment combinations. Such temperatures are representative of intertidal environments on the coast of British Columbia, especially between the late autumn and early spring and along the northern coast, in Haida Gwaii and in southern Alaska (USA), where green crab have recently expanded (Yamada et al. 2001; Howard pers comm, 2023). Testing green crab foraging behaviour in temperatures relevant to the invasion locale of interest is another component of considering context-dependency.

Our findings suggest that the addition of complexity, in the form of substrate in which prey can conceal themselves, as well as variation in prey species characteristics, can alter the predictions stemming from FREs about the ecological impacts of an invasive marine predator. We observed a transition from a strongly density-dependent to a density-independent consumer-prey relationship with the addition of substrate, at least for a preferred prey. The switch in dependence occurred when preferred prey, varnish clams, were allowed to implement a defence mechanism (i.e. burying) that they would naturally rely on, which lowered green crab consumption considerably. A key question arising from our study is whether the foraging behaviour of all consumers is similarly altered by complexity and prey variability. The answer is important because the predictions of impacts made by FREs are sometimes used when comparing consumer species (invasive vs. invasive or invasive vs. native) to gauge their potential relative impacts (Dick et al. 2014; DeRoy et al. 2020b). Thus, variation in the effect of contextual factors, such as environmental complexity and prey traits, on consumer foraging will directly affect these comparative predictions. Incorporating replicable features of the natural environment into functional response experiments seems imperative to predict more accurately the impact of invasive predators on prey populations.

Data availability

The code and data underpinning the analyses reported in this paper are deposited on Github at https://github.com/elizabethoishi/green-crab-functional-response.

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Supplementary material 1

Supplementary data

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Data type: docx

Explanation note: figures for the burial depths of varnish clams and Japanese littleneck clams in substrate, lengths of clams that were or were not consumed by a green crab for each treatment combination, and number of varnish clams and Japanese littleneck clams consumed by green crabs with consumption rates as a function of initial clam density (1, 2, 4, 6, 12, 16) for each treatment combination.

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